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2-HYDROXY-4,7-DIMETHOXY-1,4-BENZOXAZIN-3-ONE (*N-O*-ME-DIMBOA), A POSSIBLE TOXIC FACTOR IN CORN TO THE SOUTHWESTERN CORN BORER[1]

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Abstract—The southwestern corn borer (SWCB), *Diatraea grandiosella* Dyar, is a major pest of corn, *Zea mays* L., in the southern United States. The damage to corn is caused primarily by larval feeding on leaf, ear, and stem tissues. In this study, 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one (*N-O*-Me-DIMBOA) was identified by MS and NMR as present in corn whorl surface waxes. This compound has evidently not been isolated previously, but its glucoside has been reported in corn, wheat, and *Coix lachryma*. It is present in the waxes in a higher concentration than DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and 6-MBOA (6-methoxybenzoxazolinone). It was toxic to the SWCB in a stress diet, but it was less toxic to this insect than 6-MBOA when incorporated in the standard rearing diet. Nevertheless, it may have some role in the resistance of corn to the SWCB because the total surface wax content is higher in resistant lines than in susceptible lines.

Key Words—Corn, *Zea mays* (L.), southwestern born borer, *Diatraea grandiosella*, Dyar, Lepidoptera, Pyralidae, feeding resistance, 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one, *N-O*-Me-DIMBOA.

INTRODUCTION

The southwestern corn borer (SWCB), *Diatraea grandiosella* (Dyar), is a major pest of corn, *Zea mays* L., in the southern United States. It attacks corn in the vegetative and reproductive stages of plant growth. In whorl stage corn, the

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SWCB larvae feed first on the tender unfurled leaves in the whorl and later within the stem (Davis et al., 1988a,b).

High protein and low fiber content have been correlated with susceptibility of whorl-stage corn to the SWCB (Hedin et al., 1984). Later, high protein and low fiber content along with the distribution of whorl free amino acids have been associated with susceptibility to another important southern corn pest, the fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) (Hedin et al., 1990). Extensive efforts to associate feeding resistance of corn to both insects with the toxicity of some corn plant allelochemicals have been largely unsuccessful, although 6-methoxybenzoxazolinone (6-MBOA) was found present in both susceptible and resistant lines and manifested some toxicity (ED_{50} = 0.40% in the SWCB, ED_{50} = 0.50% in the FAW) to these insects (Nicollier et al., 1982; Hedin et al., 1984). 6-MBOA is the major degradation product from 4-*O*-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, which upon injury or crushing, is initially hydrolyzed enzymatically to the corresponding aglycon, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Klun and Brindley, 1966).

Although DIMBOA is reported to be more toxic than 6-MBOA to the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Klun and Brindley, 1966), we have not been able to satisfactorily test DIMBOA in diets for the ECB because it is rapidly converted to 6-MBOA after its incorporation. In any event, repeated GLC analyses for 6-MBOA in whorls of SWCB and FAW leaf-feeding susceptible (S) and resistant (R) corn lines grown in Mississippi were low and not appreciably different (Hedin et al., 1984). Thus, DIMBOA and 6-MBOA may be contributing resistance factors in corn to the SWCB, but they do not appear to be decisive factors.

A long-continuing corn breeding program at this location has led to the release of eight germplasm lines with leaf-feeding resistance to the FAW and the SWCB and several other lepidopterous insects (Davis et al., 1988a,b; Williams et al., 1989). The mechanisms of the resistance have been determined as larval antibiosis and nonpreference for both the SWCB and the FAW (Wiseman et al., 1981; Davis et al., 1989). It is expected that the same or similar chemical and/or physical factors may govern the resistance of corn to these insects.

The initial objective of the present study was to carry out chemical studies on the surface waxes of S and R lines, hoping to find differences that could be associated with insect resistance. Previously, Bianchi and Avato (1984) found that the kernels, husks, and leaves of maize plants were covered by waxes comprised of long-chain alkanes, esters, aldehydes, alcohols, acids, and sterols. In preliminary tests by us, GLC and GLC-MS analyses revealed only minor differences of these expected constituents in our S and R lines, but 6-MBOA, DIMBOA, and an apparently new benzoxazinone were found present. In this study, the structure of the benzoxazinone was deduced, its relative concentration

in several S and R lines was determined, and its toxicity to the SWCB, relative to that of 6-MBOA, was evaluated.

METHODS AND MATERIALS

Plant Material. Five SWCB leaf-feeding susceptible inbred lines (AB24E, GT106, SC229, TX601, and Va35) and five SWCB leaf-feeding resistant inbred lines (Mp496, Mp704, Mp705, Mp707, and Mp708) were used in the study. These inbred lines were grown in 1990 and 1991 in the field in a randomized complete block (RCB) design with four replications by the Corn Host Plant Research Unit (USDA-ARS) at Mississippi State, Mississippi. Corn whorl tissue was collected by replicates from plants grown to the V8–V10 stage of development (Ritchie and Hanway, 1982).

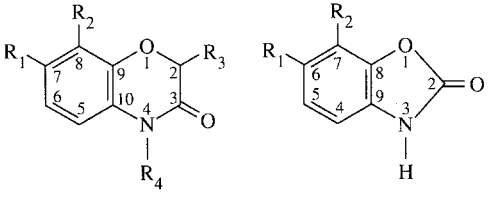
Harvesting of Surface Waxes. Surface waxes of corn whorls (later found to include 6-MBOA, DIMBOA, and related constituents) were collected by dipping and agitating corn whorl leaf sections in CH_2Cl_2 for approximately 1 min. The waxes were stored at −20°C until they were analyzed or evaluated. For GLC-MS analysis, about 200 g of each whorl sample (analyzed in duplicate) was dipped in 2 liters of CH_2Cl_2. Solubles were carefully concentrated and stored at −20°C. For preparative work to isolate adequate quantities for NMR and dietary studies, approximately 4 kg of corn whorls from two resistant inbred lines, Mp707 and Mp708, were solvent-stripped with about 40 liters of CH_2Cl_2 in batches. Yields of waxes averaged 0.047% and 0.069% from fresh tissue for S and R lines, respectively.

Dietary Constituents. The standard casein–wheat germ diet including contents and rearing procedures for the SWCB have been established by Davis (1989). The ingredients of the standard casein–wheat germ diet (used for test comparisons) and the test diet (without the casein and wheat germ) were custom prepared by Bio-Serv, Inc., Frenchtown, New Jersey. The standard diet (530 g) includes 12.6 g casein; 10.6 g wheat germ; 10.6 g agar; 36.8 g of a mixture of sucrose, salts, linseed oil, cholesterol, methyl paraben, sorbic acid, and corn cob grits; 3.7 g of a vitamin mix; 0.3 g of neomycin sulfate; and 456 ml of water. For amino acid diets, 14.17 g of a mixture of essential and nonessential amino acids were used in place of the casein and wheat germ. The contents were based on amino acid analysis of corn whorls (Hedin et al., 1990), and alphacel (alphacellulose; Bio-Serv, Inc., Frenchtown, New Jersey) was used to normalize total weight of the diet. To initiate feeding (and growth) on the amino acid diets, it was found that the addition of 1 g of wheat germ (0.2% of the diet on a wet weight basis, a nonnutritional level) was required (Hedin and Davis, unpublished data). The significance of this requirement is being investigated.

Dietary Studies. Effects of *N-O*-Me-DIMBOA (compound Ib; see Table 1) and 6-MBOA on SWCB larval growth were evaluated using a standard casein-wheat germ diet and the rearing procedure established by Davis (1989). Dietary ingredients and procedures are further listed and described by Hedin et al. (1990). Dietary evaluations were also performed using a nonoptimal synthetic amino acid diet that required the aforementional supplementation with wheat germ (0.2%) to initiate growth (Hedin and Davis, unpublished data). Each treatment was comprised of 7–17 replicates consisting of the growth of one larvae.

To test putative toxins in short supply such as 6-MBOA, 50-ml portions of the prepared diet were further mixed with 1 g of alphacel on which varying amounts of 6-MBOA had been suspended using ethyl ether as a carrier so that

TABLE 1. EI-MS FRAGMENTATIONS (m/z)[a] OF SOME 1,4-BENZOXAZIN-3-ONES AND METHOXYBENZOXAZOLINONES IN CORN WHORLS



I II

(Ia) R_1 = OMe, R_2 = H, R_3 = R_4 = OH (DIMBOA)
(Ib) R_1 = R_4 = OMe, R_2 = H, R_3 = OH (*N-O*-Me-DIMBOA)
(Ic) R_1 = OMe, R_2 = R_4 = H, R_3 = OH
(Id) No Structure Assigned
(IIa) R_1 = OMe, R_2 = H (6-MBOA)
(IIb) R_1 = R_2 = OMe

Ia 211(46), 195(28), 193(10), 180(7), 166(85), 165(100), 150(56), 138(34), 124(18), 122(12), 110(40), 106(28), 95(34)
Ib 225(48), 196(14), 193(17), 166(100), 165(95), 150(64), 138(30), 122(14), 110(42), 106(32), 95(30)
Ic 195(42), 166(100), 165(8), 138(12), 124(20), 110(14), 95(8)
Id 239(15), 225(10), 195(8), 166(34), 165(100), 150(55), 137(60), 122(18), 110(30)
IIa 166(14), 165(100), 150(58), 136(3), 122(12), 106(28), 95(16)
IIb see Ic

[a] M^+ is underlined.

the final diet contained 0.02, 0.05, 0.10, 0.20, and 0.40% of 6-MBOA on a wet weight basis. To test Ib at approximately the same level (0.25%) with only 22 and 24 mg available for two separate tests that were conducted in 1991 and 1992, 10 ml of the diet was mixed with 0.5 g of alphacel on which Ib had been suspended from ethyl ether. The above diets were poured into 45-mm-diameter crystallizing dishes to gel and 7–17 diet plugs (weighing 0.4 g each) were harvested with No. 6 cork borer. The plugs were placed in the center of a paper board cap and infested with one newly hatched SWCB larva. An inverted 30-ml plastic cup containing 2% agar was placed over the cap, which was then snapped into the rim. Similar sized diet plugs were also prepared from the control diets.

In other dietary studies, tests with 0.1 and 0.4% of 6-MBOA added to the modified synthetic amino acid diet were carried out to determine whether initiation of growth might be stimulated at relatively low levels of incorporation of 6-MBOA. Also, S and R waxes were evaluated at 0.12% of the diet for their effect on growth when added to the modified synthetic amino acid diet.

Insects. The SWCB used in these studies were obtained from laboratory colonies maintained by the Corn Host Plant Resistance Research Unit of the Crop Science Research Laboratory located at Mississippi State, using the procedures described by Davis (1989). For the growth tests, the insects were maintained at 27°C, 50–60% relative humidity, and 16:8 hr light–dark photocycle. To maintain vigor, the colony was infused with wild insects every year.

Isolation of N-O-*Me-DIMBOA (Ib).* Surface waxes of S and R lines (0.5-g aliquots) that had been collected as previously described were redissolved in hexane and chromatographed on a 2.5 × 11-cm Biosil A column (silicic acid; Biorad Laboratories, Rockville Center, New York). Fractions were eluted with 200 ml volumes of solvents of increasing polarity: CH_2Cl_2, $CHCl_3$, ethyl acetate, and methanol. The progression of the elution was monitored by silica gel TLC using $CHCl_3$–ethyl acetate 1:1 for development, and I_2 or 1% ethanolic diphenylboric acid 2-aminoethyl ester for visualization. The hydrocarbons, wax esters, and a trace of carotenes were eluted from the column with hexane and CH_2Cl_2. 6-MBOA, DIMBOA, and Ib were eluted with $CHCl_3$–ethyl acetate 1:1. Ib possessed an R_f of 0.50 (Silica gel; $CHCl_3$–ethyl acetate 1:1), while 6-MBOA (IIa) and DIMBOA (Ia) gave R_f values of 0.57 and 0.10, respectively, with the same system. Repeated chromatographic work in 1990 and 1991 yielded 22 and 24 mg, respectively, of Ib. Portions were used for gas chromatographic, spectral, and subsequently, dietary studies.

Synthesis of 6-MBOA. Quantities were available from our previous synthesis work (Nicollier et al., 1982).

^{1}HMR and ^{13}CMR Spectra. Spectra were obtained on 22.0 mg of the isolated compound (Ib) in D_6-acetone with a QE-300 GE NMR spectrometer. The spectral shifts and their assignments are given in Table 2.

TABLE 2. ^{1}H AND ^{13}C NMR SPECTRAL DATA OBTAINED FOR DIMBOA (Ia) AND A 1,4-BENZOXAZIN-3-ONE (Ib) ISOLATED FROM CORN WHORL

	^{1}H NMR (ppm)		^{13}C NMR (ppm)	
Carbon No.	Ia[a]	Ib	Ia[a]	Ib
2	5.72	5.72, s	88.1	92.5
3			208.0	205.5
5	7.25	7.18, d	109.9	107.8
6	6.68	6.73, d	103.6	103.5
7			[b]	157.2
8	6.61	6.63, d	99.2	103.0
9			137.7	137.4
10			117.8	115.9
OCH_3 (C-7)	3.77	3.80, s	51.0	55.1
OH (C-2)	2.85	3.00, s		
OCH_3 (N)		3.93, s		62.2
OH (N-4)				

[a]Campos et al. (1989).
[b]Not assigned.

Mass Spectra. Mass spectra were taken at 70 eV in the positive EI mode with a Hewlett Packard 5985B quadropole mass spectrometer. The sample was introduced into the source of the instrument via a direct insertion probe. The source was maintained at a temperature of 200°C, and the probe was ballistically heated from ambient temperature to 350°C.

Samples were also analyzed by GLC-EI-MS on a methyl silicone fused silica column (25 m × 0.25 mm, film thickness 0.25 μm, programmed from 70° to 250°C at 10°C/min, injection temperature = 200°C, detector temperature = 250°C, carrier gas: helium, 40 cm/sec) that was interfaced to the mass spectrometer. Identifications were assigned by comparison with available standards. An approximation of relative concentration of components was obtained by comparing the MS data system total abundance count of the ion chromatogram with that of appropriate standards. Multiple ion scanning was performed to determine the sequence of fragmentations, concomitantly distinguishing the presence of any isomers.

Statistical Methods. Data on larval weights were subjected to ANOVA. Means were separated using least significant differences $P < 0.05$ test. The statistical design used to compare larval growth on diets was completely random. Each treatment was comprised of 7–17 replicates consisting of the growth of one larvae, as previously stated.

RESULTS AND DISCUSSION

When the isolated surface waxes were chromatographed on a Biosil A column as described in the Methods and Materials section, elution with $CHCl_3$–ethyl acetate 1 : 1 gave a white substance on freeze-drying that could later be crystallized from ethanol or aq. ethanol. In succeeding summers, 22 and 24 mg of a benzoxazinone were collected for structural and dietary studies. The spectral data are summarized in Table 1 and Table 2 and pertinent structures are included.

EI-MS via solid probe of the benzoxazinone (Ib, Table 1) gave an apparent moleculer ion of M^+ 225 with a fragmentation pattern somewhat similar at the lower masses to DIMBOA (Ia) and 6-MBOA (IIa). The mass spectral data listed in Table 1 for 6-MBOA were reported by us previously (Nicollier et al., 1982) and that for DIMBOA was also obtained by us, but has not previously been reported. The MS data for DIMBOA is generally consistent with that reported by Chen and Chen (1976), Venis and Watson (1978), Lyons et al. (1988), and Campos et al. (1989), except that it contained some *m/e* 195 ($M^+ - 16$), which presumably can be attributed to contamination of the DIMBOA with either 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (Ic) or 6,7-dimethoxy-2-benzoxazolinone (IIb). The latter previously was found in corn by Tipton et al. (1967). Finally, evidence was obtained for another component (presumably a benzoxazinone) with an apparent M^+ of 239.

^1H NMR and ^{13}C NMR spectral data for DIMBOA (Ia) and Ib are listed in Table 2. It was possible to reconcile the spectral data reported by Campos et al. (1989) for DIMBOA with those of our isolate Ib for C-2, C-3, C-5, C-6, C-8, C-9, and C-10; the OCH_3 (C-7); and the OH (C-2). Additionally, the purified Ib gave a ^1H shift (three protons) at 3.93 ppm and a ^{13}C NMR shift at 62.2 ppm, both presumptive for an *O*-Me function. The possibility of a C-methyl on the aromatic ring is rejected because the C-5, C-6, and C-8 carbons and their protons have been established, leaving only the C-2 hydroxyl as an alternative methoxylation site to the hydroxy on the ring *N*. Methoxylation of the C-2 OH is unlikely, because of the much lower established ^1H shifts for the methoxy functions of 3.45 ppm in methoxyacetone and of 3.48 ppm in 2-methoxycyclohexanone. Evidence for *N*-OH is lacking by comparison with formaldoxime which gives a singlet at 5.15 ppm. Also, the higher ^1H shift of the *N*-OCH_3 (3.66 ppm) in *N*,*N*-acetylmethoxyaniline (Ito et al., 1980) supports assignment of the *N*-OCH_3. Hence, the structure of Ib is deduced to be 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one.

Further evidence for the *N*-*O*-Me function is provided by NMR data from the work of Nagao et al. (1985), who isolated the 2-*O*-glucoside of Ib and determined its absolute configuration. They list the ^1H NMR and ^{13}C NMR shifts as H 3.89 and 55.4 ppm respectively, both very close to our measurements.

As indicated above, the glycoside of Ib has previously been reported. Hofman and Hofmanova (1970) reported the glucoside of Ib from corn, wheat, and the roots of *Coix lachryma*. In a review article, Gambrow et al. (1986) studied the metabolic fate of Ib glucoside in wheat in which the trivial name HDIBOA-Glc was employed. Nagao et al. (1985) analyzed the stereochemistry of the Ib glucoside in *Coix lachryma* and found it to be 2-*O*-β-Glucopyranosyl-4,7-dimethoxy-1,4-(2H)-benzoxazin-3-one. Neimeyer (1988), in a review article, referred to Ib as HDMBOA-Glc. He further cited the work of Gambrow et al. (1986), who reported that Ib could not be obtained in pure form because of its ease of decomposition. In this present work, multimilligram quantities were isolated with relative ease, probably because the source was the waxy surface layer of the whorls. A review of GLC-MS data collected in this laboratory over a several-year period from whorl extracts, pressed whorl juice, and corn callus failed to reveal any evidence for *N*-*O*-Me-DIMBOA, while 6-MBOA was prominent. Because the wax layer constitutes a relatively small percent of the total whorl tissue, its content in these tissues escaped detection. Evidently, this successful isolation of *N*-*O*-Me-DIMBOA can be attributed to its stability in the wax layer.

The contents of *N*-*O*-Me-DIMBOA, DIMBOA, and 6-MBOA of the waxes from several S and R corn whorl samples as determined by GLC-FID analysis are listed in Table 3. These compounds eluted in an order that was unexpected based on molecular weights; *N*-*O*-Me-DIMBOA; 9.8 min; DIMBOA, 11.0 min; and 6-MBOA, 12.6 min. The unexpected relatively early elution of DIMBOA actually was that of the thermally dehydrated species (*m/e* 193) as later shown by GLC-MS. However, the molecular ion of DIMBOA (M^+ 211) survives solid probe MS, and *m/e* 193 ($M^+ - 18$) is a relatively minor fragment (Table 1).

The total surface wax content, which includes a number of hydrocarbons, esters, and acids in addition to *N*-Me-DIMBOA, DIMBOA, and 6-MBOA, was 46% higher (statistically significant) in resistant lines than in susceptible lines (Table 3), consistent with the visible appearance of resistant whorls, which appear to have a heavier coat of surface waxes. The content (milligrams per gram of whorl) of DIMBOA and *N*-*O*-Me-DIMBOA in resistant waxes appeared to be generally higher than in susceptible lines, while 6-MBOA appeared to be higher in susceptible lines, but the differences were not statistically significant.

Finally, dietary tests were carried out to evaluate the toxicity of *N*-*O*-Me-DIMBOA to SWCB larvae. Table 4 summarizes results from three tests in which larvae were fed on diet plugs for 7 or 13 days. Test A showed that *N*-*O*-Me-DIMBOA was not toxic to insects fed the standard casein–wheat germ diet at the same general level at which 6-MBOA appeared to decrease growth. A semilog extrapolation of data obtained by feeding five levels of 6-MBOA (0.02–0.40%) as incorporated in the standard diet gave an ED_{50} = 0.25% for 6-MBOA, similar to our previous finding of ED_{50} = 0.40 (Nicollier et al., 1982) for the SWCB.

TABLE 3. CONTENT OF *N-O*-Me-DIMBOA, AND 6-MBOA IN SURFACE WAXES OF S AND R CORN WHORLS—GLC ANALYSIS[a]

Line	Waxes (%)	Content (mg/g whorl)		
		N-O-Me-DIMBOA	DIMBOA	6-MBOA
Susceptible				
AB24E	0.048	1.28	0.38	0.16
GT106	0.55	4.82	0.91	0.36
SC229	0.043	1.15	0.74	1.52
TX601	0.046	3.63	1.80	1.16
Va35	0.045	1.61	1.40	1.80
Average	0.047 ± 0.004	2.50 ± 1.47	1.05 ± 0.50	1.00 ± 0.64
Resistant				
Mp496	0.059	8.16	1.71	0.90
Mp704	0.090	3.22	1.17	0.57
Mp705	0.078	11.34	4.10	0.73
Mp707	0.058	3.15	0.52	0.17
Mp708	0.062	[b]	[b]	[b]
Average	0.069 ± 0.013	6.46 ± 3.47	1.88 ± 1.35	0.59 ± 0.27

[a] Mean ± standard deviation, analysis was on a fresh weight basis.
[b] Sample was lost.

Test B evaluated *N-O*-Me-DIMBOA with the dietary nitrogen being supplied by amino acids rather than the casein–wheat germ so as to invoke dietary stress. While SWCB larvae will not grow on a diet in which a synthetic amino acid mixture based on the amino acid analysis of corn whorls is the sole source of protein, FAW larvae grow and emerge to adults on the same diet, although their growth is initially retarded (Hedin et al., 1990) (many other insects can also be reared on amino acid diets). This lag period has been attributed to metabolic adaptation of the young larvae to the free amino acid source. SWCB larvae grew about 30% as well during the first 13 days (32.5 mg) on the described amino acid diet fortified with 0.2% wheat germ, but on the further addition of 0.25% *N-O*-Me-DIMBOA, growth was abolished (1.6 mg). On the other hand, S and R waxes at the 0.12% level did not affect the growth of SWCB larvae on the amino acid diet fortified with 0.2% wheat germ. Finally (test C), addition of 0.1 and 0.4% 6-MBOA to the synthetic amino acid diet in the absence of 0.2% wheat germ had no further negative (or positive) effect on growth.

In summary, the structure of a new benzoxazinone, *N-O*-Me-DIMBOA, found in the surface wax of corn, was deduced, although its glucoside had previously been reported, and some evidence for the presence of two other benzoxazinones was also obtained. This component appears mainly in the surface wax along with DIMBOA and 6-MBOA. *N-O*-Me-DMBOA is present in

TABLE 4. EFFECTS OF 6-MBOA, *N-O*-Me-DIMBOA, AND WHORL SURFACE WAXES ON LARVAL GROWTH OF SOUTHWESTERN CORN BORER

Protein source[a]	Additives	Mean larval weight (mg) days after infestation 7	13
Test A			
C-WG	—	9.6	
C-WG	0.20% 6-MBOA	7.7	
C-WG	0.25% *N-O*-Me-DIMBOA	12.30	
LSD 0.5 value		(3.0)	
Test B			
C-WG	—		104.7
SYN AA	0.2% WG		32.5
SYN AA	0.2% WG + 0.12% S Wax		30.6
SYN AA	0.2% WG + 0.12% R Wax		26.8
SYN AA	0.2% WG + 0.25% *N-O*-Me-DIMBOA		1.6
LSD 0.05 value			(17.2)
Test C			
C-WG			94.4
SYN AA			3.0
SYN AA	0.1% 6-MBOA		6.1
SYN AA	0.4% 6-MBOA		3.7
LSD 0.05 value			(12.6)

[a]C-WG = casein-wheat germ, SYN AA = synthetic amino acids (Hedin et al., 1990)

corn surface waxes in higher concentration than DIMBOA and 6-MBOA. *N-O*-Me-DMBOA was toxic when incorporated in a stress (amino acid) diet, but it was less toxic than 6-MBOA to the SWCB when incorporated in the standard laboratory diet. Methoxylation of the ring *N* may contribute to its relative stability in the wax and to its limited toxicity. Nevertheless, it may have some role in the resistance of corn to the SWCB given that the surface wax content of R lines is higher than in S lines. An explanation for the effect of *N-O*-Me-DIMBOA on larval growth in stressed diets may be that hydroxamic acids inhibit lepidopteran chymotrypsin (Houseman et al., 1992).

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